

THE PROTHORACIC GLANDS OF LEUCOPHAEA MADERAE (ORTHOPTERA)

BERTA SCHARRER¹

Department of Anatomy, University of Colorado Medical Center

INTRODUCTION

Of the insect organs which furnish developmental hormones the corpora cardiaca-allata and the neurosecretory centers of the brain have been known for some time to be operative in a number of orders. More recently the endocrine role of another organ of internal secretion, the prothoracic glands, has been demonstrated in *Lepidoptera*. In this group of insects an active principle produced by these glands is necessary for pupation and imaginal differentiation, and apparently also for molting (Fukuda, 1940, 1941; Williams, 1947). These observations suggest that the factor produced by the prothoracic glands should be classified as a growth and differentiation hormone (see Scharrer, in press).

In a recent publication Williams (1948) discussed the still meager histological data available at present in regard to the prothoracic glands of certain *Lepidoptera*. In the same paper he mentioned the existence of prothoracic glands in the larva of *Cimbex americana* (*Hymenoptera*), and suggested that the organs described as "ventral glands" in *Dixippus morosus* (*Orthoptera*) by Pflugfelder (1938, 1939) may be homologous to the prothoracic glands of the *Lepidoptera*. Aside from these data there is little morphological evidence of the occurrence of prothoracic glands in insects other than *Lepidoptera*.

Since the existing experimental evidence suggests an important role of the prothoracic glands, it appears desirable to study their occurrence among various groups of insects, their histology and appearance in different phases of the life cycle, and their relationship to other endocrine organs. The investigation reported here concerns the histology of the prothoracic glands of *Leucophaea maderae* (*Orthoptera*) in different stages of postembryonic development and their involution after metamorphosis.

MATERIAL AND METHODS

The observations are based on sectioned tissue (74 specimens) and on supra-ventrally stained material (over 60 specimens). The animals used were various nymphal instars in different phases of the intermolt period, as well as male and female imagoes of *Leucophaea maderae* ranging from one hour to 15 months of "adult age." In the freshly opened insect the prothoracic glands are very difficult to see on account of their transparency which does not permit their differentiation from neighboring structures. The identification of the paired organ is greatly facili-

¹The work was done while the author held a Fellowship from the John Simon Guggenheim Foundation. Support by grants from the American Cancer Society and The Anna Fuller Fund is gratefully acknowledged.

tated by the use of supravital dyes with which the prothoracic glands stain conspicuously. Specimens having received injections of neutral red or methylene blue solutions prior to dissection offer a suitable material for the study of certain morphological features of the gland. For permanent preparations the prothoracic region was fixed in Zenker-formol, Bouin, or Carothers solution. Paraffin sections 3-7 micra thick were stained with the following techniques: Hematoxylin-eosin, Mallory-azan, Masson's and Lendrum's trichrome, and Wilder's reticulum stains. Isolated glands were incubated at 56° C. in 2 per cent osmic acid solution for several hours and were studied in toto or after paraffin embedding.²

OBSERVATIONS

The prothoracic glands of *Leucophaea* consist of two bands of tissue which occupy a ventral position in the anterior thorax in close proximity to the prothoracic ganglion. The two bands cross in the manner indicated in Figure 1; at the point of

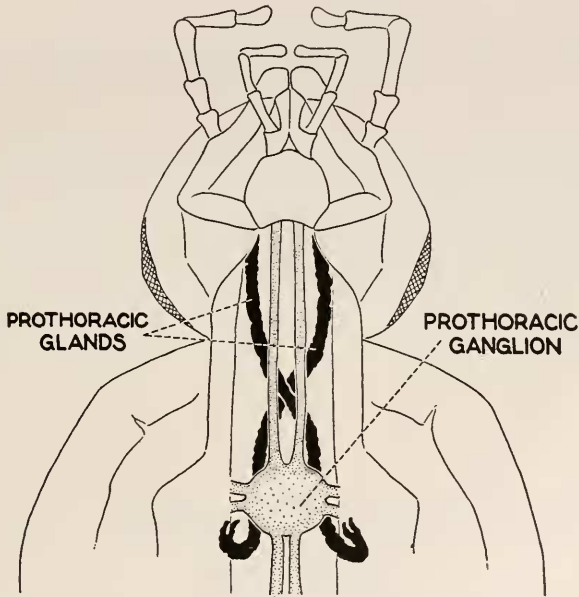


FIGURE 1. Ventral aspect of anterior thorax and head (tilted back) of *Leucophaea* nymph showing position of prothoracic glands.

crossing the organs are connected by a narrow tissue bridge. The anterior ends of the tissue bands taper off in the neck region. At the posterior end each band divides into two short branches one of which establishes nervous connection with the prothoracic ganglion. Figure 2, based on methylene blue preparations, shows a branch of a thin nerve entering the prothoracic gland shortly after its emergence from the lateral surface of the ganglion. No ducts are found in connection with

² I am indebted to Miss Kate Gruen for valuable technical assistance.

these glands; they are surrounded by blood spaces. It may be assumed, therefore, that the secretory products are given off into the blood.

The prothoracic glands are present in both nymphs and adults, but in the latter they become considerably reduced in size and structure soon after emergence.

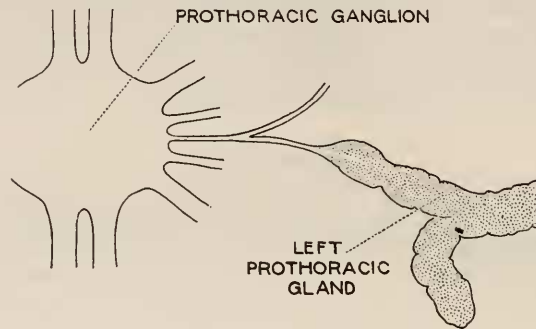


FIGURE 2. Innervation of prothoracic gland of *Leucophaea* by a lateral branch from the prothoracic ganglion, as shown for left side. Diagram based on methylene blue preparations.

A. The nymphal type

In the prothoracic gland of the nymph and of the freshly emerged adult the cells are arranged in densely packed layers around the longitudinal axis of the tissue band. The center is occupied by a trachea, a nerve, and several parallel fibers of striated muscle all of which extend throughout the length of the organ (Figs. 3, 4). The nerve is evidently derived from the prothoracic ganglion, since no other nervous

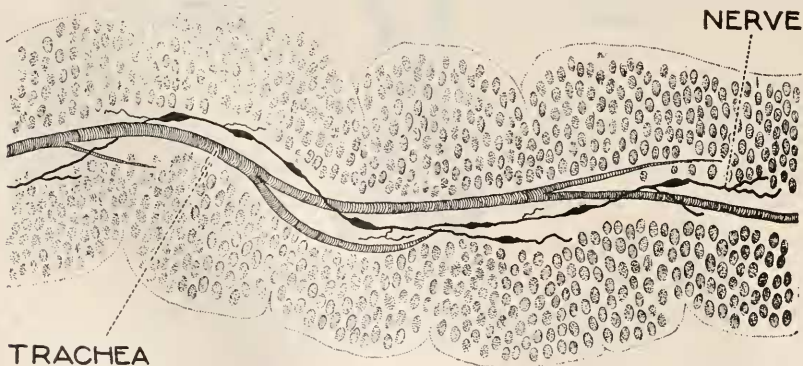


FIGURE 3. Prothoracic gland of nymph of *Leucophaea* stained supravivally with methylene blue.

branch than the one mentioned above (Fig. 2) has been observed to enter the gland. The central nerve, in addition to innervating the musculature of the organ, probably also supplies the glandular tissue; the delicate branches of the nerve could be traced only for short distances in methylene blue preparations. Due to the contraction of the axial muscle the glandular tissue in fresh and in fixed preparations is more or

less folded, with the result that the width of the organ varies. In the wider portions 8 to 12 nuclei may be counted across the width of the cellular layer of one side (Fig. 3).

The deeply staining nuclei are the most prominent feature of the gland. They are ovoid and, for the most part, approximately uniform in size and appearance. In regard to their dense arrangement and general morphology they resemble the nuclei of the corpora allata of the same species. In some nymphal glands a number of considerably larger nuclei may be observed. The majority of specimens studied showed some pycnotic nuclei in the prothoracic glands.

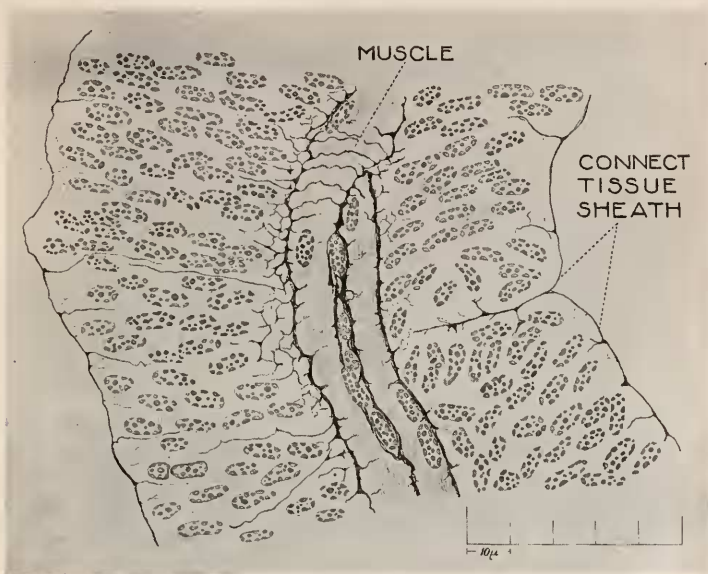


FIGURE 4. Prothoracic gland of last instar female nymph of *Leucophaea* in longitudinal section. Paraffin, 4 micra, Mallory azan.

The cytoplasm is not abundant and the cell boundaries are difficult to discern, except under favorable conditions as, for instance, in the periphery of the sectioned organ, where the nuclei are less densely packed.

With the methods employed for permanent preparations no appreciable amount of cytoplasmic inclusions indicating a secretory activity of the prothoracic gland cells was observed. In two specimens a few acidophilic granules were seen, in others the cytoplasm showed vacuolization. These observations suggest the possibility that certain cytoplasmic inclusions may be dissolved during the ordinary embedding and staining procedure. This view is further supported by the results of supravital staining. The cytoplasm of fresh tissue subjected to supravital dyes for a suitable period of time contains inclusions, as indicated in Figure 5. Round bodies varying in diameter stain a distinct blue with methylene blue. The staining is not always uniform throughout the particle; more deeply stained areas may be differentiated from a lighter background. The granular inclusions found to stain red with neutral

red are the same as those taking up methylene blue. This can be demonstrated by placing a methylene blue treated organ in a drop of weak neutral red solution on a slide. When a cover slip is used, the blue stain soon disappears from the granules and is replaced by a red tint. The same individual granules can be observed as they change color.

After incubation with osmic acid the cells of the prothoracic glands show blackened inclusions which, on account of their size and distribution, seem to be identical with the granules appearing in supravitaly stained organs. These observations suggest that the granular inclusions are lipid in nature. Further than that no definite conclusions can be drawn. On the one hand, these bodies may be interpreted as



FIGURE 5. Glandular portion of prothoracic gland of last instar nymph of *Leucophaea*, supravitaly stained, showing granular inclusions (solid black) and nuclei (in outline).

cytological manifestations of a secretory activity of the prothoracic glands, since it is known that certain types of secretion granules stain with neutral red. On the other hand the inclusion bodies may be identified as Golgi material on the basis of their similarity, both as to appearance and stainability, with the Golgi elements described in other invertebrate material (Worley, 1944).

The prothoracic glands are ensheathed by a thin connective tissue membrane from which tenuous branches may be seen to enter the glandular tissue. These branches continue into the fibrous network surrounding the muscle elements in the center (Figs. 4, 6). These relationships are best observed in azan preparations where the fibrous elements stain a bright blue.

The cells of the prothoracic glands undergo mitotic divisions. Not all specimens studied showed mitoses. In a group of 44 dated nymphs, ranging from instar four to eight, 4 micra serial sections in the horizontal plane through the prothoracic glands were checked for mitotic figures. Specimens fixed immediately after molting, or at intervals up to six days following a molt, had no mitoses; neither did nymphs preparing for a new molt as indicated by the separation of the old cuticle from the epidermis. However, a certain number of the prothoracic glands from animals fixed during the remainder of the intermolt period, showed mitotic figures. While some of these specimens had low values (approximately one mitosis in ten sections), others had an average of up to eight mitoses per section. Since the intervals between two molts in *Leucophaea* are subject to considerable variation,

no more precise conclusion can be drawn than that at some time during the intermolt period a probably short, but considerable spurt of mitotic activity takes place in the prothoracic glands.

B. Adult type

In the male and female imago of more than eight days of "adult age" the prothoracic glands are considerably changed in appearance (Fig. 7). The tissue bands have become thin and the microscopic examination shows that they consist almost exclusively of the muscular core. Most, if not all, of the glandular cells have dis-

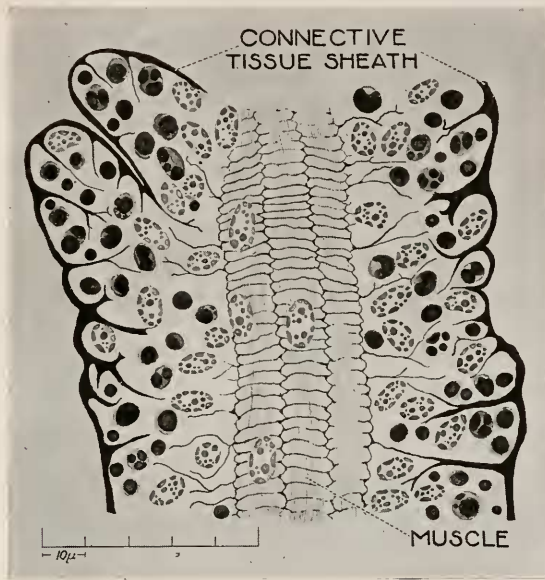


FIGURE 6. Longitudinal section of prothoracic gland of male adult *Leucophaea* four days after emergence, showing involutionary process (nuclear breakdown, decrease in width of glandular component). Paraffin, 4 micra, Mallory azan.

appeared. The connective tissue elements are more conspicuous than in the nymphal gland. This change of morphological appearance of the prothoracic glands within the short period of transition from the nymphal to the adult life may be interpreted as an indication that in the imago these organs are no longer functionally active. The steps of this striking involutionary process were traced in a series of adult male and female specimens, fixed at daily intervals after emergence. While the prothoracic glands of the freshly emerged adult resemble the nymphal organ, the first signs of regression may be noticed after 24 hours of adult life. At this stage a small number of the nuclei of the glandular component are pycnotic. Within the next three or four days the cellular breakdown becomes very conspicuous in that a large proportion of pycnotic nuclei or cellular remnants are interspersed with a gradually decreasing number of seemingly still normal nuclei (Fig. 6). The glandular tissue decreases in width, while no apparent change takes place with re-

spect to the muscular component. Animals fixed six, seven, or eight days after emergence show the involution of the prothoracic glands almost completed, practically all of the glandular tissue having disappeared by this time. From this stage on throughout adult life the organs show the same picture of involution (Fig. 7).



FIGURE 7. Longitudinal section (at point of crossing) of remnant of prothoracic glands of male adult *Leucophaea* one month after emergence. Note disappearance of glandular component, thickening of connective tissue sheath. Paraffin, 4 micra, Mallory azan.

DISCUSSION

The description given in the preceding paragraphs requires a few words of discussion of the place the organ in question occupies with reference to corresponding (homologous and analogous) structures. There can be little doubt that the prothoracic glands of *Leucophaea* are homologous with those of the *Lepidoptera*. Both are located in the anterior thorax. As in *Leucophaea* the prothoracic glands of certain *Lepidoptera* are more or less branched, band-like structures without ducts (Toyama, 1902/03; Lee, 1948). In both, the paired glands are composed of epithelioid cell elements with densely packed nuclei and a small amount of cytoplasm.

The innervation of the prothoracic glands does not represent a pertinent criterion for their identification, since a considerable variation seems to exist even within the *Lepidoptera*. In this group of insects the prothoracic glands may receive fibers

from the subesophageal ganglion, from the prothoracic ganglion, from the mesothoracic ganglion, and from the connectives between these ganglia (Lee, 1948). The glands of *Leucophaea* are innervated by fibers from the prothoracic ganglion, an observation which is not at variance with Lee's observations in *Lepidoptera*.

The prothoracic glands of *Lepidoptera* are morphologically well developed and are active as endocrine organs only in the immature insect; their presence has not been observed in the adult moth (Williams, 1948). In *Leucophaea* likewise the glandular component of the organ regresses after the emergence of the adult.

A feature characteristic of the prothoracic glands of *Leucophaea* is the presence of striated muscular tissue in the center of the organ. No such elements have been described in the corresponding organs of other insects with the possible exception of the "parenchymatous tracheal organs" of *Nepa* (p. 195). The physiological significance of this muscle is unknown. It is possible that it is instrumental in discharging the secretory product from the organ. This interpretation is suggested by the occurrence of muscular elements in exocrine glands, such as the Malpighian vessels of certain insects, likewise long, thin structures whose content is propelled towards the alimentary canal by a longitudinal muscle (Palm, 1946). Among vertebrates, contractile elements in the cytoplasm of the myoepithelial (basket) cells of certain glands (salivary, mammary, sweat, oral glands, etc.) also are thought to facilitate the expulsion of secretory products.

Another parallel exists in the occurrence of myoid cells (reticulum cells with striated fibrous elements) in the thymus of a number of vertebrates (see Bargmann, 1943). Their morphological similarity with the striated components of the prothoracic glands of *Leucophaea* is of interest in view of other features the prothoracic glands of insects and the vertebrate thymus appear to have in common (p. 195).

Whereas the homology between the prothoracic glands of *Leucophaea* and of *Lepidoptera* is reasonably well established, it is not always easy to recognize from the descriptions in the literature which of various organs in the head and thorax of different insects correspond to the prothoracic glands. Williams postulated that the "ventral glands" (Pflugfelder, 1938) of *Dixippus* (*Orthoptera*) are homologous with the prothoracic glands of *Lepidoptera*. In this connection it is highly interesting that Pflugfelder (1947) in a more recent publication extended his study of the ventral glands to include a number of insect orders, i.e., *Odonata*, *Ephemerida*, *Plecoptera*, *Saltatoria*, *Phasmida*, *Dermaptera*, *Blattaria*, *Mantodea*, and *Isoptera*. According to these data there exist in almost all lower *Pterygota* glandular organs of an endocrine character which, in their topography and histology, show many similarities. These phylogenetically ancient ventral glands are assumed to be derived from originally segmental organs whose function was excretory. In some cases the connection with the place of origin, i.e., the ventral epidermis, is still evident. More specifically, the ventral glands developed from the ectodermal canal of their precursor organs in the respective segment. During this transformation the lumen of the excretory duct gradually disappeared, a process which is indicated by the presence of a vestigial lumen in the ventral glands of *Plecoptera* and *Dermaptera*. The wall of the duct became the endocrine tissue.

It is of interest that a similar transformation from nephridial organs to glands of internal secretion occurred in certain crustaceans. The rudimentary antennal gland of the isopod *Asellus*, a serial homologue of the ventral glands of insects, is endocrine in nature (Pflugfelder, 1947).

Another recent publication must be considered in a discussion of the problem of homology of the prothoracic glands. Casal (1947) described organs in *Aeschna* (*Odonata*) as "massifs ectodermiques intersegmentaires" which, at least with respect to their component situated in the posterior head region, seem to correspond to Pflugfelder's ventral glands. With the prothoracic glands of *Lepidoptera* and *Orthoptera* (*Leucophaea*), Casal's organs have in common their occurrence in the thorax, their paired, elongated, irregularly lobated structure, and their histological appearance. Like the prothoracic glands of *Leucophaea*, the "intersegmental organs" of *Aeschna* consist of modified epithelial cells with scanty cytoplasm and closely packed nuclei and exhibit a typical cyclic behavior. A quiescent phase (after each molt) is followed by an active phase (preceding each molt), during which the nuclei show mitotic and pycnotic pictures. A "crisis" (crise cinétique) occurs in the adult which leads to the involution of the organ, comparable to that of the prothoracic glands of other insects, especially of *Leucophaea*.

An additional point of agreement exists between the observations in *Aeschna* and those reported in the present paper. The structure of the "intersegmental or-

TABLE I

	Antennal glands	1st maxillary glands	2nd maxillary glands	Thoracic nephridia		
				1	2	3
Crustaceans	Present, rudimentary, or absent (endocrine in <i>Asellus</i>)	Rudimentary, or absent	Present, rudimentary, or absent	Absent or present (Branchiura); without canal in Ostracoda	Absent or rudimentary (Ostracoda)	Absent or rudimentary (Ostracoda)
Onychophora	Rudimentary	Salivary glands	Present	Present	Present	Present
Diplopoda	Absent	Absent	Tubular glands	Absent	Absent	Absent
Chilopoda	Absent	Absent	Salivary glands?	Absent	Absent	Absent
Insects:						
<i>Apterygota</i>	Absent	Absent	Cephalic nephridia	Absent	Absent	Absent
"Lower" <i>Pterygota</i>	Absent	Corpora allata?	Ventral glands	Absent	Absent	Absent
"Higher" <i>Pterygota</i>	Absent	Corpora allata?	Salivary glands, spinning glands? prothoracic glands (<i>Bombyx</i>)	Prothoracic glands?	Absent	Absent

gans" according to Casal is identical with that of the corpora allata of the same species. The striking similarity between these endocrine organs and the prothoracic glands in *Leucophaea* has been discussed (p. 189). Comparable observations in his material and a study of the literature led Pflugfelder (1947) to suggest that the corpora allata, like the ventral glands, may be derived from nephridial organs.

If we accept this derivation as correct, the ventral glands of the lower *Pterygota* (like the cephalic nephridia of the *Apterygota* and the second maxillary glands of crustaceans) can be considered as serial homologues of the corpora allata of pterygote insects, as well as of the antennal glands of crustaceans. No derivatives of thoracic nephridial organs are known to exist in the insect orders possessing ventral glands. In all holometabolous insects, in *Hemipteroidea*, in *Mantis*, and perhaps in *Blatta*, organs corresponding to the ventral glands of other insects studied by Pflugfelder are said to be absent. However, in some of these forms (*Lepidoptera*, *Hymenoptera*, *Blattaria*) organs situated in the thorax, the prothoracic glands ("hypostigmatic glands" of Toyama, "intersegmental organs" of Casal) appear to take the place of the ventral glands. Furthermore, it is possible that a reinvestigation of the enigmatic "parenchymatous tracheal organs" in the thorax of the *Hemipteran Nepa* (Hamilton, 1931) may link these organs with the prothoracic glands of other insects.

The possible homologies of the prothoracic glands are summarized in Table I, which is based largely on Pflugfelder's data concerning the developmental history of nephridial derivatives in the *Articulata*.

With respect to *Bombyx*, Toyama (1902/03) showed that in the embryo the prothoracic glands develop from the second maxillary segment and, due to a shortening of this segment in the course of development, subsequently become located in the anterior thoracic region. If a corresponding derivation can be demonstrated in other forms possessing prothoracic glands these glands can be considered homologous with the ventral glands. However, it is quite possible that in certain insects the prothoracic glands may be shown to be derivatives of nephridia of the first thoracic segment, in which case they would be serial homologues of the ventral glands. The variability in the innervation of the prothoracic glands which may be supplied from ganglia of several segments (Lee, 1948; see also Pflugfelder, 1947) would thus be better understood.

The striking similarities in the development, structure, and life history of the ventral glands, the intersegmental organs, and the prothoracic glands suggest a correspondence in function (see Table II). It seems justified, therefore, to consider them as homologous organs whose endocrine function appears to be concerned with the control of developmental processes, both embryonic and postembryonic.

It has been mentioned before (p. 193) that the prothoracic glands of *Leucophaea* and their homologues also have some features in common with the thymus of the vertebrates; they are listed in Table III. The most interesting of these parallelisms are the involutory process at the onset of sexual maturity, and the hastening of this regression by allatectomy³ and hypophysectomy respectively.

The significance of such comparable traits is admittedly conjectural. However, it may be pointed out that there exist other structural and functional correlations between certain organs and organ systems of invertebrates and vertebrates, which

³ These observations will be reported in greater detail in a later publication.

TABLE II

	Occurrence	Location	Morphology	Histology	Life history	Histological cycles relative to molting	Function
Ventral glands	Lower <i>Pterygota</i> (Plugfelder '38, '39, '40, '47)	Ventrocaudal head region	Paired, elongate, lobated, originally with duct	Epithelioid cells of ectodermal origin, occasional very large cells (Saltatoria); structure similar to that of corpora allata	Presence in immature stages (embryonic and larval); regression in adults: nuclear fragmentation and degeneration (pycnosis); no regression in termite workers and soldiers	Secretory granules in intercellular spaces; mitoses before (last) molt and nuclear fragmentation	Assumed endocrine (regulation of development); dependency on corpora allata
Intersegmental organs (massifs ectodermiques intersegmentaires)	<i>Odonata</i> : <i>Aeschna</i> (Cazal '47)	Posterior head, thorax	Paired, elongate, lobated; intersegmental invagination continuous with epidermis	Epithelioid cells, dense nuclei, little cytoplasm; structure identical with that of corpora allata	Presence in immature stages, regression in adults ('crise cinétique')	Mitoses and pycnoses preceding molts, quiescent period following molts	Assumed control of nucleoprotein metabolism, comparable to lymphoid elements (thymus)
Prothoracic glands (hypostigmatic glands, Toyama)	<i>Lepidoptera</i> (Toyama '02/03, Ke '30, Fukuda '40, '41, Williams '47, '48, Lee '48)	Prothorax, mesothorax	Paired, elongate, lobated or branched, without duct	Large epithelioid cells, little cytoplasm	Presence in embryos, larvae and pupae, absence in adults		Endocrine: source of growth and differentiation hormone(s) regulating molting, pupation, and adult differentiation
	<i>Hymenoptera</i> : <i>Cimbex</i> (Williams '48)	Prothorax			Presence in larvae		
	<i>Orthoptera</i> : <i>Leucophata</i>	Prothorax	Paired, elongate, without duct; good nervous and tracheal supply; muscular component	Epithelioid cells, little cytoplasm, occasional large cells; structure like that of corpora allata	Presence in nymphs, regression in adults	Cyclic mitotic activity	Assumed endocrine; dependency on corpora allata
Parenchymatous tracheal organs (?)	<i>Hemiptera</i> : <i>Nepa</i> (Hamilton '31)	Thorax	Muscular nature, rich tracheal supply		Regression in adults		Unknown

TABLE III

Prothoracic glands (and equivalents)	Thymus
Location in posterior head and thorax	Location in neck and thorax
Derived from bilateral invaginations of ectoderm	Derived from bilateral epithelial ingrowths
Ducts originally present, later rudimentary or absent	Ducts reduced to rudiments during development
Segmental development	Segmental derivation (from several pharyngeal pouches)
Syncytial structure (<i>Platysamia</i>)	Syncytial reticulum
Lymphocyte-like cell components (<i>Bombyx</i>)	So-called thymocytes of lymphocyte type
Muscular components (<i>Leucophaea</i>)	Myoid cells
Maximum size before emergence of adult, involution in imago	Maximum relative size before puberty, involution after puberty (higher vertebrates)
Influenced by endocrine disturbances (involution hastened by allatectomy, <i>Leucophaea</i>)	Sensitive toward endocrine imbalance, for instance hypophysectomy
Endocrine function established in <i>Lepidoptera</i> (control of developmental processes)	Doubtful endocrine function related to growth and development

cannot be dismissed as superficial and accidental similarities, but which indicate similar principles of functional organization. Thus the intercerebralis-cardiacum-allatum system of insects is analogous to the hypothalamo-hypophyseal system of vertebrates (Hanström, 1941; Scharrer and Scharrer, 1944), the internephridial organs of the worm *Physcosoma* to the interrenal body (Harms, 1921), the x-organ of crustaceans to the thyroid, and the sinus gland of crustaceans to the paraphysis (Hanström, 1941). From these examples as from many others the concept evolves ever more clearly that the gap between invertebrates and vertebrates has in the past been magnified out of its true proportions.

SUMMARY

1. The prothoracic glands of *Leucophaea maderae* are paired band-like structures located in close proximity to and innervated by the prothoracic ganglion. The longitudinal axis contains striated musculature, a nerve and a trachea. The glands are well developed in nymphal stages, but regress in the imago.

2. In the nymph the glandular tissue consists of layers of dense nuclei surrounded by scanty cytoplasm. In their histological appearance the prothoracic glands strikingly resemble the corpora allata of the same species. The nymphal prothoracic glands exhibit a cyclic nuclear activity, characterized by a spurt of mitotic divisions during the intermolt period and by quiescent phases preceding and following each molt.

3. In freshly emerged male and female adults the prothoracic glands are still nymphal in appearance. Involution takes place during the first week of the adult stage. It manifests itself by a breakdown of nuclei and results in a reduction of the tissue bands to practically nothing except the muscular core.

4. The prothoracic glands of *Leucophaea* are considered to be homologous with the prothoracic glands of *Lepidoptera* and *Hymenoptera*, with the "ventral glands" of lower *Pterygota*, with the "intersegmental organs" of *Odonata*, and possibly with the "parenchymatous tracheal organs" of *Hemiptera*. They have certain features in common with the thymus of the vertebrates.

LITERATURE CITED

- BARGMANN, W., 1943. Der Thymus. *Handb. mikr. Anat. Mensch.*, edited by W. v. Moellendorff, 6: part 4, pp. 1-172.
- CAZAL, P., 1947. Recherches sur les glandes endocrines retrocérébrales des insectes. II. Odonates. *Arch. zool. expér. gén.*, 85: 55-82.
- FUKUDA, S., 1940a. Induction of pupation in silkworm by transplanting the prothoracic gland. *Proc. Imp. Acad. Tokyo*, 16: 414-416.
- FUKUDA, S., 1940b. Hormonal control of molting and pupation in the silkworm. *Proc. Imp. Acad. Tokyo*, 16: 417-420.
- FUKUDA, S., 1941. Role of the prothoracic gland in differentiation of the imaginal characters in the silkworm pupa. *Annot. Zool. Japon.*, 20: 9-13.
- HAMILTON, M. A., 1931. The morphology of the water-scorpion, *Nepa cinerea* Linn. (Rhynchota, Heteroptera). *Proc. Zool. Soc. London*, 1931: 3 and 4, pp. 1067-1136.
- HANSTRÖM, B., 1941. Einige Parallelen im Bau und in der Herkunft der inkretorischen Organe der Arthropoden und der Vertebraten. *Lunds Univ. Årsskr. N. F. Avd. 2*, 37: no. 4, 1-19.
- HARMS, W., 1921. Morphologische und kausalanalytische Untersuchungen ueber das Internephridialorgan von *Physcosoma lanzarotae* nov. spec. *Arch. Entwickl. Mech.*, 47: 307-374.
- KE, O., 1930. Morphological variation of the prothoracic gland in the domestic and the wild silkworms (In Japanese with English summary). *Bulteno Scientia Fakult. Terkult. Kyushu Imp. Univ. Fukuoka*, 4: 12-21.
- LEE, T. Y., 1948. A comparative morphological study of the prothoracic glandular bands of some lepidopterous larvae with special reference to their innervation. *Ann. Ent. Soc. Amer.*, 41 (in press).
- PALM, N. B., 1946. Studies on the peristalsis of the malpighian tubes in insects. *Lunds Univ. Årsskr. N. F. Avd. 2*, 42: no. 11, 1-39.
- PFLUGFELDER, O., 1938. Weitere experimentelle Untersuchungen ueber die Funktion der *Corpora allata* von *Dixippus morosus* Br. *Zeit. wiss. Zool.*, 151: 149-191.
- PFLUGFELDER, O., 1939. Wechselwirkungen von Druesen innerer Sekretion bei *Dixippus morosus* Br. *Zeit. wiss. Zool.*, 152: 384-408.
- PFLUGFELDER, O., 1947. Ueber die Ventraldruesen und einige andere inkretorische Organe des Insektenkopfes. *Biol. Zentralbl.*, 66: 211-235.
- SCHARRER, B. Hormones in insects. *Hormones, chemistry, physiology, and clinical applications*, edited by G. Pincus and K. V. Thimann, vol. I (in press).
- SCHARRER, B., AND E. SCHARRER, 1944. Neurosecretion VI. A comparison between the intercerebralis-cardiacum-allatum system of the insects and the hypothalamo-hypophyseal system of the vertebrates. *Biol. Bull.*, 87: 242-251.
- TOYAMA, K., 1902/03. Contributions to the study of silk-worms. I. On the embryology of the silk-worm. *Bull. Coll. Agric. Tokyo Imp. Univ.*, 5: 73-118.
- WILLIAMS, C. M., 1947. Physiology of insect diapause. II. Interaction between the pupal brain and prothoracic glands in the metamorphosis of the giant silkworm, *Platysamia cecropia*. *Biol. Bull.*, 93: 89-98.
- WILLIAMS, C. M., 1948. Physiology of insect diapause. III. The prothoracic glands in the *Cecropia* silkworm, with special reference to their significance in embryonic and post-embryonic development. *Biol. Bull.*, 94: 60-65.
- WORLEY, L. G., 1944. Studies of the vitally stained Golgi apparatus. III. The methylene blue technique and some of its implications. *J. Morph.*, 75: 261-289.